

AMENDMENTS TO THE SPECIFICATION

Please replace the first and second full paragraphs on page 17 of the specification with the following amended paragraphs:

Construction of plasmid pSIPI

To place the resolution sites at the desired positions in the SIP vector it was necessary to introduce suitable restriction sites in plasmid pSIP to enable directional cloning of the resolution sites. Plasmid pSIP was linearised with HindIII and subsequently digested with NaeI. The resulting 2995 bp HindIII / NaeI fragment was eluted from an agarose gel. This fragment contains the regulatory elements of the araBAD operon and the essential elements for self immobilisation. A 3131 bp-DNA fragment containing the β -lactamase gene and origins of replication of M13 and of pBR322 was generated by PCR-amplification (extension time: 7 min) using the primers pSIPI5 (5'-CAGCAGAAGCTTGT TTTTGGCGG- ATGAGAGAAG-3') (SEQ ID NO: 1) and pSIPI3 (5'-AGATCTCTGCTGGCGGCCGCGGTTGCTGGC- GCCTATATC-3') (SEQ ID NO: 2) and the vector pSIP as template. Primer pSIPI5 contains a single HindIII site, pSIPI3 contains a BglII and a NotI restriction site. After digestion of the PCR-fragment with HindIII it was ligated with the 2995bp fragment described above via their blunt site obtained by the Pfu polymerase and the corresponding HindIII site, respectively, resulting in the vector pSIPI. Due to the cloning strategy following restriction sites were introduced: SacII, NotI, BglII, Ball.

Construction of plasmid pSIPIres

The resolution sites were generated by PCR amplification (extension time: 1 min) using the vector pJMS11 as template and the following primer sets: resolution site 1: 5res1: 5'-

CAGCAGCTGCAGCCTTGGTCAAA-TTGGGTATACC-3' (SEQ ID NO: 3); 3res1: 5'-CTGCTGAAGCTTGCACATATGTGGGCGTGAG-3' (SEQ ID NO: 4); resolution site 2: 5res2: 5'-CAGCAGGCGGCCGCCCTTGGTCAAATTGGGTATACC-3' (SEQ ID NO: 5) 3res2: 5'-CTGCTGAGATCTGCACATATGTGG GCGTGAG-3' (SEQ ID NO: 6). The PCR-fragment encoding the resolution site 2 contained a unique NotI site at the 5' end and a BglII site at the 3' end and was cloned into the corresponding single sites of pSIP1 resulting in the vector pSIP1res2. The resolution site 1 fragment containing a single PstI site at the 5' end and HindIII site at the 3' end, respectively, was then cloned into the corresponding single sites of pSIP1res2 resulting in the vector pSIP1res.

Please replace the first and second paragraphs on page 18 of the specification with the following amended paragraphs:

Construction of plasmid pSIP1resHCN

The pBR322 origin of replication in the vector pSIP1res was replaced by the high copy number origin of replication from plasmid pUC19. Therefore the pUC19 origin sequence was amplified by PCR (extension time: 2 min) from pUC19 using primers 5ori (5'-CAGCAGGCCGGCTGAGCAAAAGGCCAGCA-3') (SEQ ID NO: 7) and 3ori (5'-TGCTGCGCGGCCGCTAGAAAAGATCAAAGGATCTTCTTGAG-3') (SEQ ID NO: 8). The 5ori primer contains a single NaeI site and the 3ori primer a single NotI site. After digestion of the PCR-fragment with NaeI and EagI it was cloned into the corresponding sites of a 4828bp NaeI/EagI-fragment derived from vector pSIP1res via partial digestion with NaeI and EagI, resulting in the vector pSIP1resHCN (HCN: HighCopyNumber).

Construction of plasmid pKLysBADparA

A 1920 bp DNA fragment encoding the parA gene under expression control of the arabinose operon was amplified using plasmid pBADparA as template and the primers BADKLysfw (5' - ATTCCGACTAGTCAAGCCGTCAATTGTCTG 3') (SEQ ID NO: 9) and BADKLysrev (5' - AGCCCTAGATCTTTATTTTGCTGCTGCGC 3') (SEQ ID NO: 10) containing terminal SpeI and BglII sites, respectively (extension time: 4 min). The DNA fragment was cloned into the two corresponding sites of the broad host range and low copy plasmid pKLysparAS derived from pKLys36. In this construct (pKLysBADparA) the E-specific lysis cassette of plasmid pKLys36 is flanked by the parA resolvase gene, which is under transcriptional control of the arabinose inducible PBAD promoter (Fig. 2).

Please replace the first full paragraph on page 20 of the specification with the following amended paragraph:

Example 3

Minicircle DNA immobilised in Escherichia coli ghosts

Construction of plasmid pHCNparA and pBADparA

A 672-bp PCR fragment containing the parA resolvase gene was generated by PCR-amplification (extension time: 2 min). Plasmid pJMSB8 was used as template and primers BADparAfw (5'-ATAGAACCATGGCGACGCGAGAGCAACAAC 3') (SEQ ID NO: 11) and BADparArev (5'-AGCCCTCTGCAGTTATTTTGCTGCTGCGC 3') (SEQ ID NO: 12) to introduce NcoI and PstI restriction sites, respectively. The PCR fragment was cloned into the corresponding sites of plasmid pSIPresHCN, resulting in plasmid pHCNparA (Fig. 5) and into pBAD24 resulting in pBADparA.

Please replace the first paragraph on page 21 of the specification with the following amended paragraph:

Construction of plasmid pSIPHCNparA

Using plasmid pBAD24parA as template a 672-bp PCR fragment, which codes for the parA resolvase and the ribosomal binding site derived from pBAD24, was obtained by PCR-amplification (extension time: 2 min). Oligonucleotides HCNparAfw (5'-ACCGAACTGCAGCTACACCATACCCGTTTTTTT-GGGC 3') (SEQ ID NO: 13) and HCNparArev (5'-AGCCCTCTGCAGAAGCTTTTATTTTGCTGCTG-CGC 3') (SEQ ID NO: 14) containing PstI and PstI/HindIII as terminal restriction site were used as primers. The fragment was digested with PstI and cloned into the corresponding sites of plasmid pSIPresHCN, resulting in plasmid pSIPHCNparA (Fig. 6).